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PHASE 7, EFFECT OF DERMAL APPLICATIONS OF N,N-DIETHYL-META-TOLU--ETC(U)
FEB 81 R A ANGERHOFER, M H WEEKS
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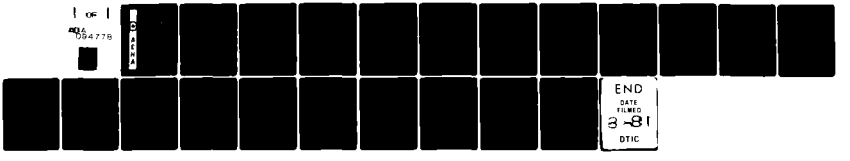

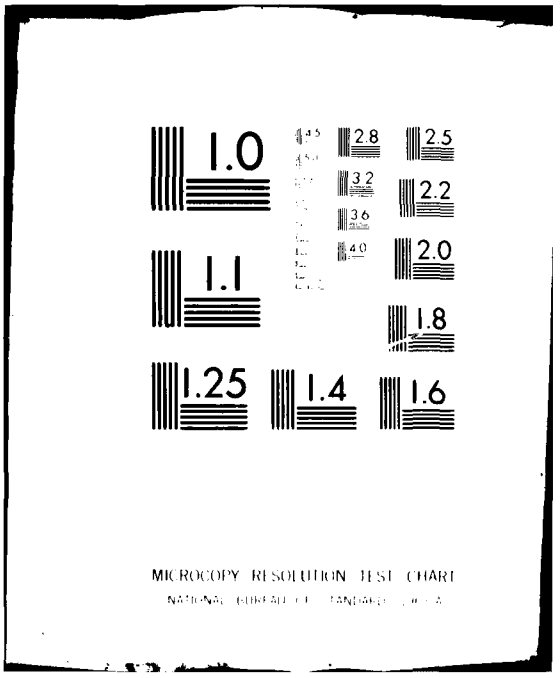
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# UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

## ABERDEEN PROVING GROUND, MD 21010

(6)

PHASE 7,
EFFECT OF DERMAL APPLICATIONS OF N,N-DIETHYL-META-TOLUAMIDE (M-DET) ON THE EMBRYONIC DEVELOPMENT OF RABBITS,
~~STUDY NO. 75-51-0034-81-7~~
MARCH 1979 - JULY 1980,

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Co Richard A. Angerhofer
Maurice H. Weeks



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| 4. TITLE (and Subtitle) Phase 7, Effect of Dermal Applications of N,N-Diethyl-meta-toluamide (m-Det) on the Embryonic Development of Rabbits, Study No. 75-51-0034-81, March 1979 - July 1980 | | 5. TYPE OF REPORT & PERIOD COVERED<br>Phase 7, Mar 79 - Jul 80<br>6. PERFORMING ORG. REPORT NUMBER |
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Insect repellent
m-Det
N,N-Diethyl-meta-toluamide
Skin irritation
Teratology

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Daily dermal applications of an ethanol solution of m-Det were administered to pregnant rabbits from day 1 through day 29 of gestation. Dosages were selected as fractions of the dermal $LD_{50}$ for that species (50, 100, 500 and 1000 mg/kg/day corresponding to 1/100, 1/50, 1/10 and 1/5 of the $LD_{50}$).
Repeated applications of a 75 percent solution of m-Det caused moderate to severe skin irritation in rabbits with the degree of irritation dependent upon the daily dosage. It would be expected to cause irritation to human skin upon repeated applications at high concentrations if the skin is left unwashed.

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Item 20. ABSTRACT (continued).

All maternal and fetal indices were within normal limits. The single abortions occurring in each of the highest two dosage groups was presumed to be spontaneous events caused by handling the animals or the discomfort of the skin irritation.
M-Det was not shown to be a teratogen in rabbits and would not be expected to cause birth defects in humans.

DEPARTMENT OF THE ARMY
U.S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Weeks/lm/AUTOVON
584-3980

HSE-LT/WP

9 FEB 1981

SUBJECT: Phase 7, Effect of Dermal Applications of N,N-Diethyl-meta-toluamide (m-Det) on the Embryonic Development of Rabbits, Study No. 75-51-0034-81, March 1979 - July 1980

Executive Secretary
Armed Forces Pest Management Board
Forest Glen Section
Walter Reed Army Medical Center
Washington, DC 20012

A summary of the pertinent findings and recommendations of the inclosed report follows:

a. Daily dermal applications of an ethanol solution of m-Det were administered to pregnant rabbits from day 1 through day 29 of gestation. Dosages were selected as fractions of the dermal LD-50 for that species (50, 100, 500 and 1000 mg/kg/day corresponding to 1/100, 1/50, 1/10 and 1/5 of the LD-50).

b. Repeated applications of a 75 percent solution of m-Det caused moderate to severe skin irritation in rabbits with the degree of irritation dependent upon the daily dosage. It would be expected to cause irritation to human skin upon repeated applications at high concentrations if the skin is left unwashed.

c. All maternal and fetal indices were within normal limits. The single abortions occurring in each of the highest two dosage groups were presumed to be spontaneous events caused by handling the animals or the discomfort of the skin irritation.

d. M-Det was not shown to be a teratogen in rabbits and would not be expected to cause birth defects in humans.

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CONTENTS

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP

# PHASE 7
# EFFECT OF DERMAL APPLICATIONS OF N,N-DIETHYL-META-TOLUAMIDE (M-DET) ON THE EMBRYONIC DEVELOPMENT OF RABBITS
# STUDY NO. 75-51-0034-81*†
# MARCH 1979 - JULY 1980

## 1. AUTHORITY.

a. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, Agricultural Research, Science and Education Administrations; titled Coordination of Biological and Toxicological Testing of Pesticides, effective January 1979.

b. Letter, Armed Forces Pest Control Board, 17 March 1977, subject: Registration Data for N,N-Diethyltoluamide Repellent.

2. REFERENCES. See Appendix A for a listing of references and Appendix B for infrared spectrum of N,N-Diethyl-meta-toluamide.

3. DEFINITIONS. A list of specialized terms is found in Appendix C.

4. PURPOSE. The purpose of this study was to determine the potential, if any, for N,N-Diethyl-meta-toluamide to cause embryotoxicity or defects in the developing rabbit fetus. This study was designed according to the 1966 "Guidelines for Reproductive Studies for Safety Evaluation of Drugs for Human Use" distributed by FDA. Data, consisting of maternal and fetal observations, was derived from impregnated rabbits treated dermally throughout the gestation period with this commonly used insecticide.

5. BACKGROUND. M-Det is an insect repellent widely used in both military and private sectors as a topical or clothing treatment. According to Federal Specification O-I-503E, the repellent is available in both 50 and 75 percent solutions with ethanol. These are much higher concentrations than are available on the civilian market. Gleibermann, et al (reference 4, Appendix A) reported that dermally applied diethyltoluamide at doses of 1000 mg/kg/day caused embryo and neonatal toxicity in white rats. In light of these findings, it was considered advisable to conduct a teratological investigation in a second species.

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* In conducting the study described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education and Welfare Publication No. (NIH) 78-23, revised 1978.
† The experiments reported herein were performed in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

6. PROCEDURE.

a. Test Materials. The test material used in this study was N,N-Diethyl-meta-toluamide (m-Det, DEET, DETA) with a minimum meta isomer content of 95 percent and 5 percent maximum of other isomers. The material (Lot No. 7141) was manufactured by Hardwicke Chemical Company, Elgin, SC 29045, and packaged for McLaughlin Gormly King Company, 8810 Tenth Avenue North, Minneapolis, MN 55427.

b. Animals. New Zealand White rabbits were visually mated twice with the same proven male at Dutchland Farms, Denver, PA. Four separate orders of thirty animals each were obtained at approximately 45-day intervals. Upon arrival at this Agency on the day of mating, each rabbit was identified numerically and placed in separate dosage groups using a table of random numbers. Groups were identified by written card, ear mark and color code. All animals received food (Wayne 15 percent Rabbit Ration, Allied Mills, Inc.) and water ad libitum during the gestation period. Rabbits were housed in individual cages, 18x16x24 inches. The room temperature was maintained at 21 $\pm$ 1°C and humidity was kept between 35-45 percent.

c. Dosing Method. Each delivery of thirty rabbits was divided into six groups of five animals each. The experimental design appears as Table 1. All rabbits were clipped free of hair dorsally from the scapular to the sacral area immediately upon arrival (16 hours before the initial application of m-Det) and weekly thereafter if needed. Dosages were selected as fractions of the dermal $LD_{50}$ for technical grade material in rabbits (5000 mg/kg). The four dosage levels of m-Det were applied daily from day 1 of gestation through day 29 of gestation on skin areas of up to 150 $cm^2$. M-Det was inuncted over the shaved area, burdened with a screened foam pad which was secured in place with cloth surgical tape. The covering was designed in such a way as to allow for an air space over the treated area while preventing the animals from ingesting the compound (reference 8, Appendix A). Doses were applied to the back through the screen using a blunted hypodermic needle. All compound was applied as a 75 percent solution of the previously described m-Det in absolute ethanol. Foam dressings were changed when needed.

d. Sacrifice Schedule. All animals were sacrificed with overdoses of barbiturates administered intravenously on day 30 of gestation. Any rabbit dying spontaneously during the course of the study was submitted for gross necropsy within 12 hours of death.

e. Observations. Skin irritation scores and body weights were recorded weekly throughout the gestation period. All toxic manifestations were recorded. Photographs of skins of representative animals from all dermally dosed groups were taken periodically during the study.

Study No. 75-51-0034-81, Mar 79-Jul 80

TABLE 1. DOSAGE GROUPS

| Dosage Group | No. Rabbits Each Shipment | Total Rabbits for Study |
|---|---|---|
| I. Solvent Control (1.33 ml/kg/day Abs. ETOH) | 5 | 20 |
| II. Untreated (Cage) Control | 5 | 20 |
| III. 1/5 Dermal $LD_{50}$ (1000 mg/kg/day, m-Det) | 5 | 20 |
| IV. 1/10 Dermal $LD_{50}$ (500 mg/kg/day, m-Det) | 5 | 20 |
| V. 1/100 Dermal $LD_{50}$ (50 mg/kg/day, m-Det). | 5 | 20 |
| VI. Estimated Human Usage (100 mg/kg/day, m-Det) | 5 | 20 |

f. Clinical Chemistry Tests. Arterial blood samples were taken from the ears of test animals for clinical chemistry tests on days 1, 7, 14, 21 and 30 of gestation (test days 0, 6, 13, 20 and 29). The following clinical parameters were analyzed for in the serum: triglycerides, gamma glutamyl transpeptidase (GGTP), blood urea nitrogen (BUN), total protein, alkaline phosphatase, total bilirubin, glucose, lactic dehydrogenase (LDH), cholesterol, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), sodium, potassium and calcium. Determinations were made using an Abbott Bichromatic Analyzer 100® or an Instrumentation Laboratories 143 Digital Flame Photometer® (potassium and sodium).

g. Necropsy. The ovaries and uterus of each doe were exposed by laparotomy. Counts were made of corpora leutea, implantation sites and viable fetuses. The uterine position of each fetus was diagrammed and fetal numbers were assigned depending upon position. Each fetus was then weighed, measured for crown-rump length, tagged and observed for gross abnormalities. Even numbered fetuses were placed in Bouin's fixative for subsequent soft tissue examination while odd numbered fetuses were placed in 95 percent ethanol for processing for skeletal examination. The placenta of each live fetus was also weighed. Sections of skin, ovaries and uterus were taken from each doe and submitted for histopathological examination.

h. Skeletal Examinations. Ethanol-fixed fetuses were eviscerated and prepared for examination by the method of Dawson (reference 7, Appendix A). The stained fetuses were examined for variations and malformations while suspended in 100 percent glycerin.

i. Soft Tissue Examinations. Bouins-fixed fetuses were examined for visceral anomalies by razor blade cross sections after the method described by Wilson and Warkany (reference 6, Appendix A).

j. Data Evaluation. Wilson's one way nonparametric analysis of variance was used to detect differences between groups for each test day. Spearman's nonparametric correlation was used to evaluate dose-response trends for the same data. If differences were significant beyond the 0.05 level of probability, multiple nonparametric Mann-Whitney "U" tests were used to compare each combination of two groups to locate those significantly different than the others. Trends were also evaluated by reviewing graphs of the raw data to insure that no changes in dispersion or frequency of occurrence of outliers effected the results. Where indicated, a Students "t" test was used to evaluate data. Except for food consumption data, nonpregnant female values were not included in the statistical evaluations.

7. SUMMARY OF TEST RESULTS.

a. Maternal Parameters.

(1) Clinical Picture of Females. All animals were received in good condition although in a wider weight range than desired. Health and attitude were generally good throughout the study.

(a) When several animals appeared to be anorexic, daily measurement of food consumption was initiated. A statistically significant trend toward lower food consumption was noted among the higher m-Det dosage groups during the first 2 weeks of gestation in comparison with the ethanol controls. Six rabbits apparently starved at the 1000 mg/kg dosage level as well as one at 500 mg/kg and one in the ethanol control group.

(b) Dermal irritation including erythema, cracking and edema was seen in all female rabbits in the higher m-Det dosage groups (100, 500, 1000 mg/kg) by the 7th day of application. In the lowest dosage group (50 mg/kg), similar irritation was noted by the 14th day. Irritation persisted and increased in area and became somewhat more severe as the study progressed.

(2) Maternal Body Weights. All groups showed consistent body weight gains during the course of the study. No significant differences were detected when comparing the m-Det treated groups with the ethanol controls.

(3) Necropsy and Histopathological Findings. No dose related differences were found when comparing the fertility index, implantations per doe and fetuses per doe among the groups. Except for dermal irritation in the m-Det treated rabbits, no gross lesions were found at necropsy. Upon microscopic examination of treated skin, dose related degrees of hyperkeratosis, parakeratosis, and acanthosis were seen. No lesions were seen in untreated or ethanol treated skin. No exposure related lesions were seen in the ovaries or uteri.

(4) Abortions. One doe in each of the higher dosage groups (500 and 1000 mg/kg) aborted its litter before termination of pregnancy.

(5) Clinical Chemistry. Analysis of blood samples as described previously revealed significant dose related changes in GGTP and BUN. The GGTP levels were elevated at days 7, 14, 21 and 30 days of gestation in animals receiving 500 and 1000 mg/kg/day of m-Det. Levels of that enzyme were also elevated at day 14 in the 100 mg/kg/day group. On the 30th day of gestation, BUN levels were elevated in animals receiving 500 and 1000 mg/kg/day of m-Det.

b. Fetal Parameters.

(1) Fetal Statistics. There were no biologically significant differences between dosage groups with respect to fetal weight, fetal length, placental weight and sex ratios. The number of resorptions per doe in the group treated with 500 mg/kg/day of m-Det was higher than in other groups, but no dose response developed for that parameter.

(2) Skeletal Examinations. Many common variations were observed randomly among fetuses from all dosage groups. These included, but were not limited to: supernumerary ribs, unossified or reduced sternebrae, and evidence of slightly advanced or retarded ossification. Minor vertebral malformations were seen in one fetus from the untreated control group and in a fetus from the 50 mg/kg/day group.

(3) Soft Tissue Examinations. No variations or malformations of biological significance were found.

8. DISCUSSION AND CONCLUSIONS.

a. Repeated applications of a 75 percent solution of N,N-Diethyl-meta-toluamide caused moderate to severe skin irritation in rabbits. It would be expected to cause irritation to human skin upon repeated applications at high concentrations if the skin is left unwashed.

b. Treatment with m-Det caused a loss of appetite in some animals in the higher dosage groups. Large hair balls were found in the stomachs of the

high dose animals which starved themselves. This is explainable in that rabbits tend to lick at irritated areas and although the treated skin was protected by a barrier, these animals were probably licking near the affected site, swallowing hair in the process.

c. All maternal and fetal indices were within normal limits. The single abortions occurring in each of the highest two dosage groups were probably spontaneous events caused by handling the animals or the discomfort of the skin irritation.

d. Previous subchronic oral dosing with m-Det in rabbits at this Agency produced elevated blood GGTP levels and fatty liver changes at a dose of 528 mg/kg for 15 days. This teratology study indicates the same type of changes from dermally applied m-Det. These changes may reflect detoxification pathways of m-Det in the liver or perhaps may serve as an indicator of intoxication.

e. No dose related fetal abnormalities were found and it appears that m-Det administered dermally throughout gestation does not disrupt fetal development in this species even at unreasonably high dosages.

9. CONCLUSION. N,N-Diethyl-meta-toluamide at the concentration tested has the potential to produce moderate to severe dermal irritation within 7 days when applied daily to unwashed skin. M-Det was not shown to be a teratogen in rabbits and would not be expected to cause birth defects in humans.

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APPROVED:

ARTHUR H. McCREESH, Ph.D.
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# APPENDIX A

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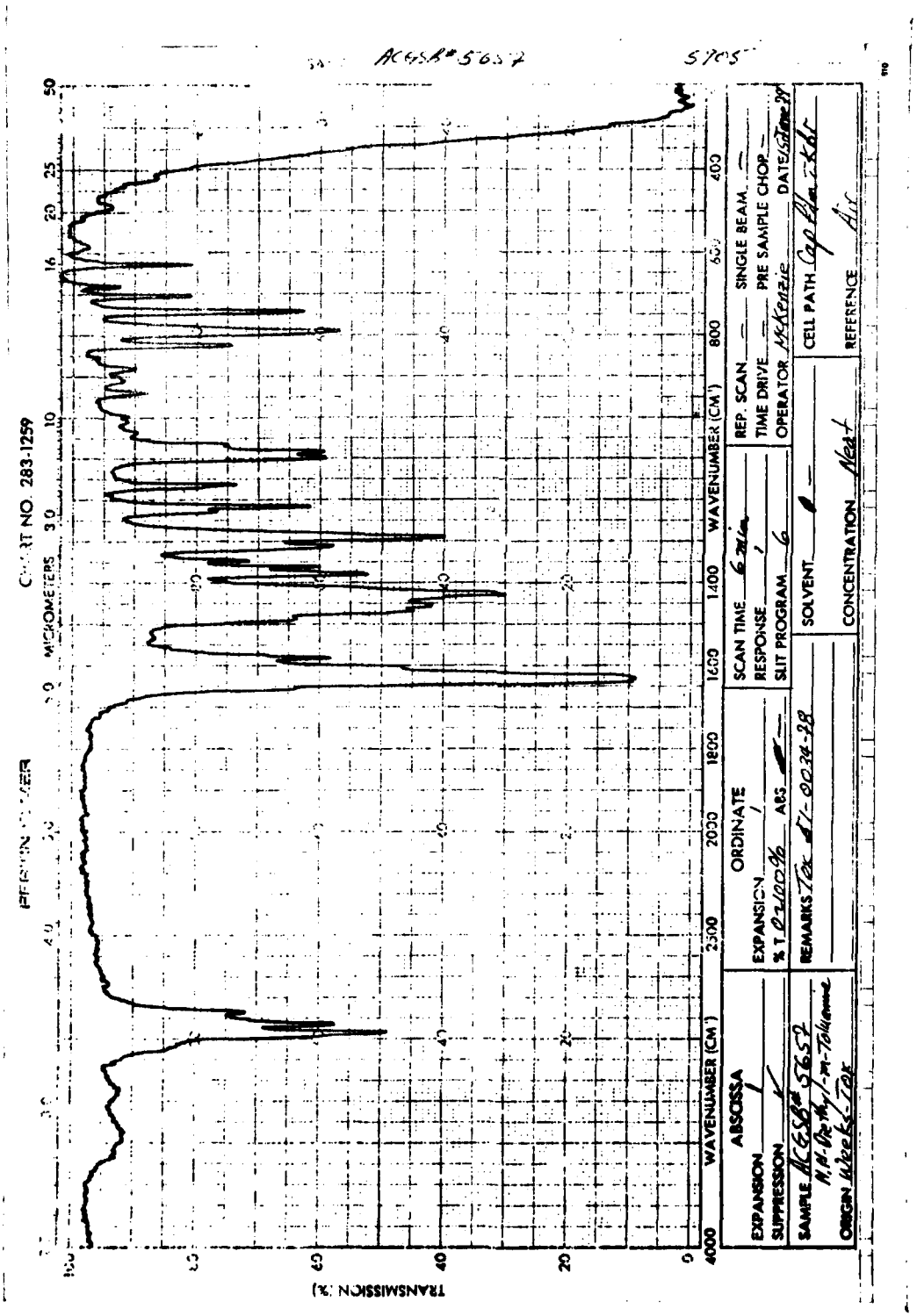
APPENDIX B

INFRARED SPECTRUM OF N,N-DIETHY-META-TOLUAMIDE



# APPENDIX C

## DEFINITIONS

**Index of fertility:** $\frac{\text{pregnant animals}}{\text{mated animals}} \times 100$

**Index of viable births:** $\frac{\text{alive normal fetuses}}{\text{total number of fetuses}} \times 100$

**Index of resorptions:** $\frac{\text{resorptions}}{\text{implantations}} \times 100$

**Early resorptions:** implantation sites (no identifiable fetal parts)

**Late resorptions:** mummifications

**Runts:** Animals weighing less than 70 percent of the average weight of the pups in that litter

APPENDIX D

SUMMARY OF MATERNAL BODY WEIGHTS (kg)

| Treatment Group (mg/kg) m-Det Applied Dermally | Gestation Day 1 | 7 | 14 | 21 | 30 |
|---|---|---|---|---|---|
| Control-ETOH | 3.45 ±0.65 | 3.68 ±0.58 | 3.83 ±0.49 | 3.97 ±0.54 | 3.95 ±0.52 |
| Control-Untreated | 3.42 ±0.60 | 3.69 ±0.64 | 3.85 ±0.62 | 4.00 ±0.65 | 4.02 ±0.56 |
| 50 | 3.41 ±0.66 | 3.58 ±0.65 | 3.70 ±0.62 | 3.89 ±0.69 | 3.85 ±0.57 |
| 100 | 3.51 ±0.47 | 3.71 ±0.41 | 3.88 ±0.40 | 3.95 ±0.49 | 3.93 ±0.43 |
| 500 | 3.36 ±0.44 | 3.50 ±0.40 | 3.59 ±0.44 | 3.70 ±0.45 | 3.74 ±0.44 |
| 1000 | 3.37 ±0.39 | 3.43 ±0.47 | 3.55 ±0.46 | 3.68 ±0.48 | 3.72 ±0.42 |

## APPENDIX E

### FOOD CONSUMPTION (g/kg/day)

| m-Det Teratology Treatment Group | | Test Day 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ETOH Control | $\bar{x}$ | 61 | 61 | 61 | 59 | 53 | 62 | 58 | 58 | 61 | 64 | 66 | 65 | 60 | 56 | 61 |
| | ± SD | ± 18 | ± 10 | ± 18 | ± 20 | ± 20 | ± 13 | ± 10 | ± 10 | ± 9 | ± 10 | ± 16 | ± 13 | ± 18 | ± 14 | ± 23 |
| Untreated | $\bar{x}$ | 46* | 53* | 54 | 57 | 56 | 57 | 51 | 60 | 63 | 63 | 62 | 55 | 52 | 52 | 56 |
| | ± SD | ± 19 | ± 11 | ± 10 | ± 10 | ± 14 | ± 15 | ± 12 | ± 17 | ± 18 | ± 19 | ± 20 | ± 14 | ± 15 | ± 12 | ± 13 |
| 1000 mg/kg/day | $\bar{x}$ | 35* | 35* | 40* | 43* | 36* | 37* | 41* | 45* | 54 | 51* | 49* | 42* | 36* | 37* | 41* |
| | ± SD | ± 20 | ± 19 | ± 19 | ± 21 | ± 18 | ± 19 | ± 14 | ± 13 | ± 12 | ± 10 | ± 12 | ± 14 | ± 16 | ± 20 | ± 18 |
| 500 mg/fg/day | $\bar{x}$ | 43* | 45* | 48 | 52 | 46 | 49* | 45* | 53 | 53 | 52* | 50* | 45* | 41* | 44* | 42* |
| | ± SD | ± 14 | ± 17 | ± 16 | ± 17 | ± 14 | ± 16 | ± 11 | ± 38 | ± 12 | ± 11 | ± 10 | ± 13 | ± 12 | ± 29 | ± 14 |
| 100 mg/kg/day | $\bar{x}$ | 51 | 51* | 56 | 54 | 49 | 53 | 52 | 52 | 54* | 52* | 55* | 53* | 49* | 47* | 49 |
| | ± SD | ± 11 | ± 12 | ± 12 | ± 10 | ± 10 | ± 11 | ± 7 | ± 11 | ± 7 | ± 9 | ± 12 | ± 10 | ± 9 | ± 7 | ± 11 |
| 50 mg/kg/day | $\bar{x}$ | 51 | 56 | 56 | 59 | 50 | 56 | 54 | 59 | 58 | 57 | 58 | 54 | 46* | 48 | 53 |
| | ± SD | ± 13 | ± 10 | ± 12 | ± 12 | ± 15 | ± 14 | ± 16 | ± 15 | ± 18 | ± 17 | ± 20 | ± 22 | ± 12 | ± 10 | ± 23 |

* Significantly different from ETOH control for that test day ($p<.05$)

## APPENDIX E

### FOOD CONSUMPTION (g/kg/day)

| m-Det Teratology Treatment Group | | Test Day 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ETOH Control | $\bar{x}$ | 57 | 58 | 59 | 59 | 47 | 40 | 46 | 46 | 41 | 37 | 32 | 34 | 35 | 38 |
| | ± SD | ±23 | ±23 | ±19 | ±24 | ± 9 | ±17 | ±21 | ±18 | ±17 | ±17 | ±18 | ±25 | ±27 | ±17 |
| Untreated | $\bar{x}$ | 61 | 57 | 61 | 53 | 48 | 45 | 40 | 41 | 36 | 41 | 36 | 42 | 39 | 43 |
| | ± SD | ±21 | ± 9 | ±15 | ±19 | ±12 | ±15 | ±10 | ±15 | ±14 | ±27 | ±20 | ±29 | ±17 | ±23 |
| 100 mg/kg/day | $\bar{x}$ | 48 | 47 | 44 | 41 | 36* | 35 | 33 | 39 | 38 | 39 | 35 | 33 | 37 | 43 |
| | ± SD | ±18 | ±20 | ±33 | ±29 | ±20 | ±19 | ±18 | ±13 | ±12 | ±14 | ±17 | ±18 | ±20 | ±18 |
| 500 mg/kg/day | $\bar{x}$ | 49 | 49 | 52 | 48 | 48 | 41 | 36 | 38 | 33 | 31 | 28 | 32 | 40 | 33 |
| | ± SD | ±12 | ±13 | ±12 | ±10 | ±15 | ±12 | ±14 | ±20 | ±14 | ±16 | ±18 | ±14 | ±26 | ±24 |
| 100 mg/kg/day | $\bar{x}$ | 54 | 48 | 52 | 46* | 43 | 43 | 36 | 30* | 28* | 28 | 30 | 30 | 30 | 37 |
| | ± SD | ±11 | ± 6 | ± 8 | ± 7 | ± 5 | ± 7 | ±13 | ±16 | ±15 | ±15 | ±15 | ±18 | ±17 | ±14 |
| 50 mg/kg/day | $\bar{x}$ | 59 | 55 | 58 | 54 | 47 | 47 | 47 | 46 | 37 | 34 | 32 | 34 | 37 | 39 |
| | ± SD | ±21 | ±15 | ±15 | ±22 | ±10 | ±17 | ±19 | ±23 | ±21 | ±24 | ±25 | ±25 | ±21 | ±21 |

* Significantly different from ETOH control for that test day ($p<.05$)

APPENDIX F

SUMMARY OF MATERNAL AND FETAL PARAMETERS

| | ETOH Control | Cage Control | 50 mg/kg | 100 mg/kg | 500 mg/kg | 1000 mg/kg |
|---|---|---|---|---|---|---|
| No. Mated | 20 | 20 | 20 | 20 | 20 | 20 |
| No. Died* | 1 | 0 | 0 | 0 | 1 | 6 |
| No. Delivered Full Term before 30th day of test* | 0 | 0 | 0 | 0 | 1 | 1 |
| No. Females Sacrificed on 30th day of test | 19 | 20 | 20 | 20 | 18 | 13 |
| Females Pregnant | 14 | 16 | 15 | 16 | 17 | 12 |
| Fertility Index (%) | 74.00 | 80.00 | 75.00 | 80.00 | 94.44 | 92.31 |
| Abortions | 0 | 0 | 0 | 0 | 1 | 1 |
| Full Term Litters | 14 | 16 | 15 | 16 | 16 | 11 |
| Gestation Index (%) | 100 | 100 | 100 | 100 | 94.12 | 91.67 |
| Implantation, Total | 112 | 133 | 127 | 143 | 141 | 91 |
| Implantations per Doe | 8.00 | 8.31 | 8.47 | 8.94 | 8.81 | 8.27 |
| Fetuses, Total | 108 | 127 | 124 | 137 | 128 | 88 |
| Fetuses per Doe | 7.71 | 7.94 | 8.27 | 8.56 | 8.00 | 8.00 |
| Alive Fetuses, Total | 106 | 127 | 124 | 137 | 128 | 88 |
| Alive Fetuses/Doe | 7.57 | 7.94 | 8.27 | 8.56 | 8.00 | 8.00 |
| Index of Alive Fetuses (%) | 98.15 | 100 | 100 | 100 | 100 | 100 |
| Dead Fetuses, Total | 2 | 0 | 0 | 0 | 0 | 0 |
| Dead Fetuses/Doe | 0.14 | 0 | 0 | 0 | 0 | 0 |
| Index of Dead and Born Fetuses (%) | 1.85 | 0 | 0 | 0 | 0 | 0 |
| Resorptions, Total | 4 | 6 | 3 | 6 | 13 | 4 |
| Resorptions/Doe | 0.29 | 0.38 | 0.20 | .38 | .81 | .36 |
| Resorption Index (%) | 3.57 | 4.51 | 2.36 | 4.20 | 9.22 | 4.40 |
| Runts | 2 | 2 | 5 | 3 | 2 | 1 |
| Avg Fetal Wt (g) | 46.14 ±8.08 | 45.16 ±7.24 | 45.63 ±7.46 | 43.58 ±9.00 | 45.94 ±8.92 | 46.90 ±8.10 |
| Avg Fetal Length (cm) | 10.03 ±1.05 | 9.87 ±0.69 | 9.95 ±0.91 | 9.89 ±0.93 | 10.05 ±0.91 | 10.26 ±0.94 |
| Avg Placental Wt (g) | 6.73 ±1.44 | 6.52 ±1.21 | 6.58 ±1.28 | 6.70 ±1.48 | 6.59 ±1.59 | 6.96 ±1.33 |
| Sex Ratios (Female/Male) | 0.89 | 1.46 | 1.31 | 0.64 | 0.56 | 1.17 |

* Numbers not used in statistical analyses.

# APPENDIX G
## Summary of Histopathological Examination

### Ethanol Controls

| Rabbit No. | Control Skin | Test Skin | Ovaries | Uterus |
|---|---|---|---|---|
| 417 | ✓ | ✓ | ✓ | ✓ |
| 418 | ✓ | ✓ | ✓ | ✓ |
| 419 | ✓ | ✓ | ✓ | X |
| 420 | ✓ | ✓ | ✓ | ✓ |
| 421 | P | ✓ | ✓ | ✓ |
| 483 | ✓ | ✓ | ✓ | ✓ |
| 484 | ✓ | ✓ | ✓ | ✓ |
| 485 | ✓ | ✓ | ✓ | ✓ |
| 486 | ✓ | ✓ | ✓ | ✓ |
| 487 | P | P | ✓ | ✓ |
| 569 | ✓ | ✓ | ✓ | ✓ |
| 570 | ✓ | ✓ | ✓ | ✓ |
| 571 | P | ✓ | ✓ | ✓ |
| 573 | ✓ | ✓ | ✓ | ✓ |
| 655 | ✓ | ✓ | ✓ | ✓ |
| 656 | ✓ | ✓ | ✓ | ✓ |
| 657 | ✓ | ✓ | ✓ | ✓ |
| 658 | ✓ | ✓ | ✓ | ✓ |
| 659 | ✓ | ✓ | ✓ | ✓ |

### Untreated Controls

| | | | | |
|---|---|---|---|---|
| 422 | ✓ | ✓ | ✓ | ✓ |
| 423 | X | X | ✓ | ✓ |
| 424 | ✓ | ✓ | ✓ | ✓ |
| 425 | ✓ | ✓ | ✓ | ✓ |
| 426 | ✓ | ✓ | ✓ | ✓ |
| 488 | ✓ | ✓ | ✓ | ✓ |
| 489 | ✓ | ✓ | ✓ | ✓ |
| 490 | ✓ | ✓ | ✓ | ✓ |
| 491 | ✓ | ✓ | ✓ | ✓ |
| 492 | ✓ | ✓ | ✓ | ✓ |
| 574 | ✓ | ✓ | ✓ | ✓ |
| 575 | ✓ | X | ✓ | ✓ |
| 576 | X | ✓ | ✓ | ✓ |
| 577 | ✓ | ✓ | ✓ | ✓ |
| 578 | ✓ | ✓ | ✓ | ✓ |
| 660 | ✓ | P | ✓ | ✓ |
| 661 | ✓ | ✓ | ✓ | ✓ |
| 662 | ✓ | ✓ | ✓ | ✓ |
| 663 | ✓ | ✓ | ✓ | ✓ |
| 664 | ✓ | ✓ | ✓ | ✓ |

Study No. 75-51-0034-81, Mar 79-Jul 80

1000 mg/kg/day M-Det

| Rabbit No. | Control Skin | Test Skin | Ovaries | Uterus |
|---|---|---|---|---|
| 431 | ✓ | 1b,2a,3c,6b | ✓ | X |
| 493 | ✓ | 1b,2a,3b,6a | ✓ | ✓ |
| 494 | P | 1b,2a,3a,6a | ✓ | ✓ |
| 495 | X | 1b,2,3b,6a | ✓ | ✓ |
| 497 | ✓ | 1a,2,3a-3b,6a | ✓ | ✓ |
| 579 | ✓ | 1b,2,3a | ✓ | ✓ |
| 580 | ✓ | 1c,2a,3b,4a | ✓ | ✓ |
| 581 | ✓ | 1b,2a,3a | ✓ | ✓ |
| 582 | ✓ | 1b,2a,3b,4 | ✓ | ✓ |
| 583 | ✓ | 1b,2a,3a,4 | ✓ | ✓ |
| 665 | ✓ | 1a,2,3 | ✓ | ✓ |
| 666 | ✓ | 1a,2,3a | ✓ | ✓ |
| 668 | ✓ | 1a,2a,3a | ✓ | ✓ |
| 669 | ✓ | 1a,2a,3b | ✓ | ✓ |

500 mg/kg/day M-Det

| | | | | |
|---|---|---|---|---|
| 433 | ✓ | 1a-1b,2,3a,6 | ✓ | ✓ |
| 434 | ✓ | 1a-1b,2,3,6 | ✓ | ✓ |
| 435 | P | 1a-1b,2,3a | ✓ | X |
| 436 | ✓ | P | ✓ | ✓ |
| 498 | ✓ | 1a,2,3a,6 | ✓ | ✓ |
| 499 | ✓ | 1b,2b,3c,6a | ✓ | ✓ |
| 500 | ✓ | 1b,2,3b,6a | ✓ | ✓ |
| 501 | ✓ | 1a,2a,3a | ✓ | ✓ |
| 502 | P | 1a,1b,2,3 | ✓ | ✓ |
| 584 | ✓ | 1a-1b,2,3a,4a | ✓ | ✓ |
| 585 | ✓ | 1a-1b,2,3a,4 | ✓ | ✓ |
| 586 | ✓ | P | ✓ | ✓ |
| 587 | ✓ | 1a,2,3a,4 | ✓ | ✓ |
| 588 | ✓ | 1a,2,3a | ✓ | ✓ |
| 670 | ✓ | 1a,2,3a | ✓ | ✓ |
| 671 | ✓ | 1,2,3 | ✓ | ✓ |
| 672 | ✓ | 1a,2,3b | ✓ | ✓ |
| 673 | ✓ | 1,2 | ✓ | ✓ |
| 674 | ✓ | 1a,2,3 | ✓ | ✓ |

Study No. 75-51-0034-81, Mar 79-Jul 80

100 mg/kg/day M-Det

| Rabbit No. | Control Skin | Test Skin | Ovaries | Uterus |
|---|---|---|---|---|
| 442 | ✓ | 1a,2a,3a-3b,6a | ✓ | ✓ |
| 443 | ✓ | 1a-1b,2a,3a | ✓ | ✓ |
| 444 | ✓ | 1a,3 | ✓ | ✓ |
| 445 | ✓ | 1a,2a,3a,6a | ✓ | ✓ |
| 446 | ✓ | 1a-1b,2,3a,6 | ✓ | ✓ |
| 508 | ✓ | 1,2,3a | ✓ | ✓ |
| 509 | ✓ | 3a | ✓ | ✓ |
| 510 | ✓ | 3a-3b | ✓ | ✓ |
| 511 | ✓ | 1,3b | ✓ | ✓ |
| 512 | ✓ | 1a,2,3a-3b | ✓ | ✓ |
| 594 | ✓ | 1a,2,3a | ✓ | ✓ |
| 595 | ✓ | 1a,2a,3a | ✓ | ✓ |
| 596 | ✓ | 1a,2a,3 | ✓ | ✓ |
| 597 | ✓ | 1b,2,3,5 | ✓ | ✓ |
| 598 | ✓ | 1b,2a,3 | ✓ | ✓ |
| 680 | ✓ | 1,2,3 | ✓ | ✓ |
| 681 | ✓ | 1a,2,3 | ✓ | ✓ |
| 682 | ✓ | 1,2,3 | ✓ | ✓ |
| 683 | ✓ | 1,2,3 | ✓ | ✓ |

50 mg/kg/day M-Det

| | | | | |
|---|---|---|---|---|
| 437 | ✓ | 1-1a,2,3a | ✓ | ✓ |
| 438 | ✓ | 1-1a,2,3 | ✓ | ✓ |
| 439 | ✓ | P | ✓ | ✓ |
| 440 | ✓ | P | ✓ | ✓ |
| 441 | ✓ | 1a-1b,2a,3b,6 | ✓ | ✓ |
| 503 | X | X | X | X |
| 504 | ✓ | 1a,2,3a | ✓ | ✓ |
| 505 | ✓ | 1a,2a,3a | ✓ | ✓ |
| 506 | ✓ | 1a,2a | ✓ | ✓ |
| 507 | ✓ | 1a,2,3 | ✓ | ✓ |
| 589 | ✓ | 1a,2,3 | ✓ | ✓ |
| 590 | ✓ | 1a,2a,3a | ✓ | ✓ |
| 591 | ✓ | 1a,2a,3b | ✓ | ✓ |
| 592 | ✓ | 1,2,3 | ✓ | ✓ |
| 593 | ✓ | 1a,2,3 | ✓ | ✓ |
| 675 | ✓ | ✓ | ✓ | ✓ |
| 676 | ✓ | ✓ | ✓ | ✓ |
| 677 | ✓ | 1,2 | ✓ | ✓ |
| 678 | ✓ | 1,2,3 | ✓ | ✓ |
| 679 | ✓ | 1,2,3 | ✓ | ✓ |

KEY

X = no tissue
P = poor section
✓ = normal

1 = Hyperkeratosis, rare, skin
1a = Hyperkeratosis, minimal, skin
1b = Hyperkeratosis, moderate, skin
1c = Hyperkeratosis, severe, skin

2 = Parakeratosis, rare, skin
2a = Parakeratosis, minimal, skin
2b = Parakeratosis, moderate, skin

3 = Acanthosis, rare, skin
3a = Acanthosis, minimal, skin
3b = Acanthosis, moderate, skin
3c = Acanthosis, severe, skin

4 = Polymorphonuclear lenkocyte infiltration, rare, keratin layer
4a = Polymorphonuclear lenkocyte infiltration, minimal, keratin layer
4b = Polymorphonculear lenkocyte infiltration, moderate, keratin layer

5 = Folliculitis, focal, minimal, subeutis, skin

6 = Mononuclear inflammatory cells, subacute, diffuse, rare, upper dermis, skin
6a = Mononuclear inflammatory cells, subacute, diffuse, minimal, upper dermis, skin
6b = Mononuclear inflammatory cells, subacute, diffuse, moderate, upper dermis, skin

Comments: No exposure related lesions were observed in the ovaries or uterus.

Similar skin lesions were seen in all four dosage groups that appeared in a dose response manner to include hyperkeratosis, parakeratosis, and acanthosis. The two higher dosage groups (1000 mg/kg and 500 mg/kg) has nutrophil infiltration into the thickened keratin layer. These cells appeared to be related to small intrakeratin layer serum pockets.

Although not noted on the microscopy table upper dermal cellularity increased in a dose response manner and was seen for all four dosage groups. The cells referred to are the normal white blood cell component of the dermis.

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